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Supplemental Information

Self-Amplifying Pulsatile Protein Dynamics without Positive Feedback

Rosa Martinez-Corral, Elba Raimundez, Yihan Lin, Michael B. Elowitz, and Jordi Garcia-Ojalvo

Figure S1. Noise effects on downward pulsing in the 3-variable negative feedback model. Related to Figure 2.

Figure S2. Effect of the parameter values on the 3-variable model dynamics. Related to Figure 2.

Figure S3. Effect of zero-order kinetics in the repressor x. Related to Figure 3.

Figure S4. Pulsing in an alternative two-variable, activator-repressor, model. Related to Figure 3.

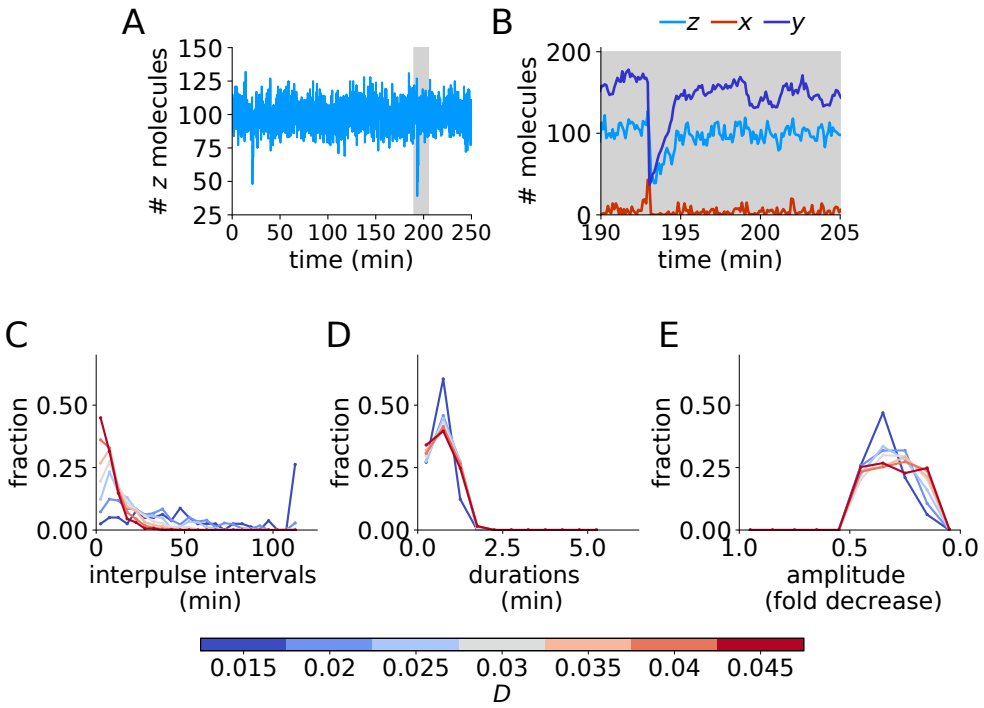


Figure S1: **Noise effects on downward pulsing in the 3-variable negative feedback model.** Related to Figure 2. A-B) Gillespie simulation shows that downward pulses in z also appear with intrinsic noise. Panel B shows the time evolution of the three variables over the grey shaded area in panel A. Simulations were performed in Stochkit [55], with the same parameters as in Figure 2D, assuming that the total number of molecules of x_{tot} and z_{tot} is 300. C-E) Effect of the noise strength D of the Ornstein-Uhlenbeck process (Eq. 12). Rest of parameters as in Figure 2D. C) Distribution of interpulse intervals. D) Distribution of pulse durations. E) Distribution of pulse amplitudes, taken as (mininum-steady state)/(steady state). Pulses were identified based on a threshold of half the deterministic steady state value for z deactivation. Dots correspond to the centre of the histogram bins, of width 5 min, 0.5 min and 0.1, respectively.

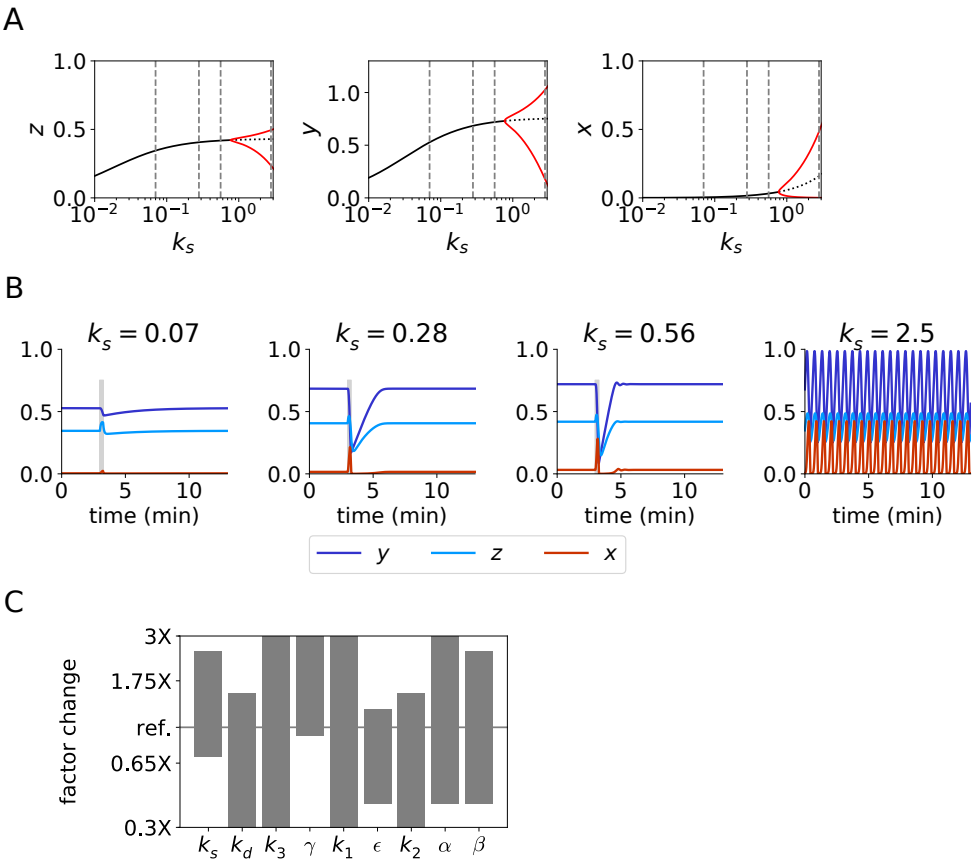


Figure S2: **Effect of the parameter values on the 3-variable model dynamics.** Related to Figure 2. A-B) Transition to oscillations as a function of k_s. A) Bifurcation diagram. Each subplot represents a variable. Dashed lines correspond to the simulations in B. B) Time traces for different k_s values. The first 3 plots show the response to a perturbation as in Figure 2C. C) Sensitivity to single-parameter value changes with respect to the parameter values of Figure 2C. We varied each parameter by a factor ranging from 0.3 to 3 fold, and assessed the ability of the circuit to respond to a perturbation in β (30% reduction during 15 s) with an inactivation pulse below 60% the steady-state value. The grey bars delimit the fold-change values for which the pulsatile response persists.

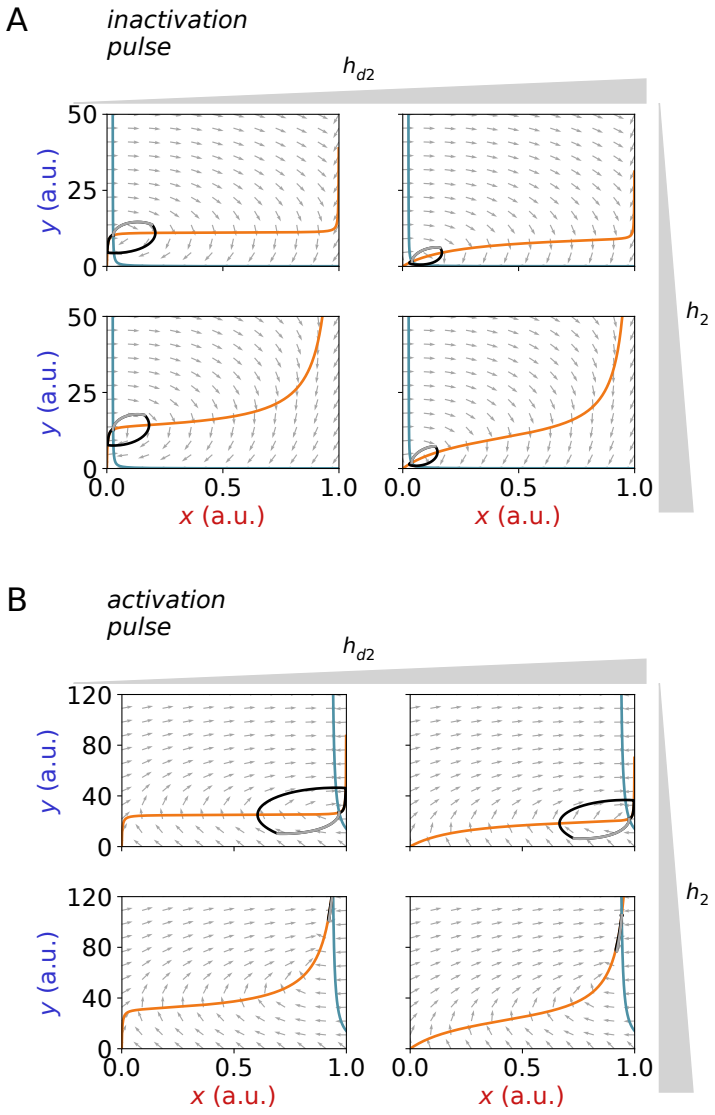
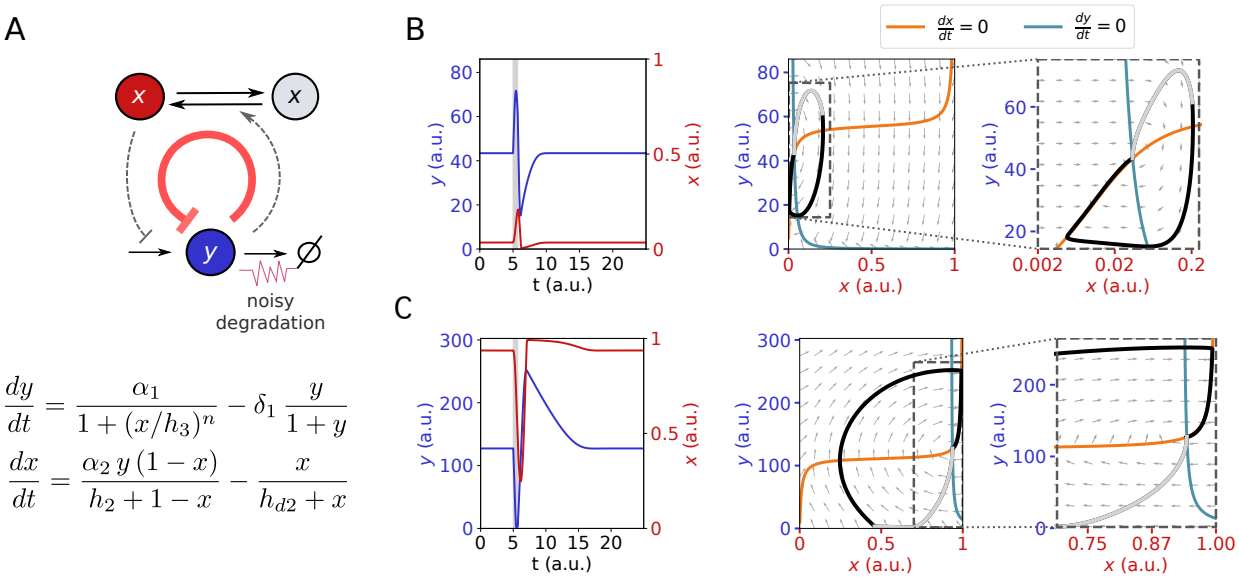


Figure S3: **Effect of zero-order kinetics in the repressor x.** Related to Figure 3. A) Inactivation pulses require saturation in x deactivation (small h_{d2}, first column). The rest of the parameters as in Figure 3B. B) Activation pulses require saturation in x activation (small h_2, first row). The rest of the parameters as in Figure 3F. Orange nullcline corresponds to Eq. 13, blue nullcline corresponds to Eq. 14. Low h_{d2}= low h_2 = 0.0025, high h_{d2} = high h_2= 0.25.



$$\frac{dy}{dt} = \frac{\alpha_1}{1+(x/h_3)^n} - \delta_1 \frac{y}{1+y}$$
$$\frac{dx}{dt} = \frac{\alpha_2\, y\,(1-x)}{h_2+1-x} - \frac{x}{h_{d2}+x}$$

Figure S4: **Pulsing in an alternative two-variable, activator-repressor, model.** Related to Figure 3. A) Scheme of the model and corresponding equations. The activator (y) activates the repressor (x), which inhibits the production of the former. B) Inactivation pulses in response to a 30% decrease in δ_1 during 0.7 time units. Parameters (in arbitrary units): $\alpha_1 = 337$, $\delta_1 = 335$, $h_3 = 0.2$, $n = 2$, $\alpha_2 = 0.018$, $h_2 = 0.01$, $h_{d2} = 0.01$. Left panel: time evolution. Shaded grey area indicates the time during which the system is perturbed. Middle panel: nullclines and trajectory on the phase-plane during the perturbation (grey line) and upon its release (black line). Arrows indicate the direction of the flow, size has been normalised for clarity and does not reflect magnitude. Right panel: inset of the region of the middle panel highlighted by a dashed square, to better appreciate the region around the fixed point. Notice that here the x axis is in log scale. C) Activation pulses in response to a 2-fold increase in δ_1 during 0.7 time units. Parameters:
$\alpha_1 = 624, \delta_1 = 335, h_3 = 1, n = 2, \alpha_2 = 0.009, h_2 = 0.01, h_{d2} = 0.01$. Panels as in B.